

composites used in the examples is provided. Carbon fibrils (Hyperion Catalysis) were compounded with poly(ethylene-co-vinyl acetate) (EVA) and the resulting composite material was extruded into sheets. The sheets were oxidized with chromic acid to expose carbon fibrils near the surface and to introduce carboxylic acid groups. Protein was immobilized on the composite by activation of the carboxylic acid groups with ethyl-dimethylpropyl-carbodiimide (EDC) in the presence of N-hydroxysuccinimide (NHS) followed by treatment with the protein in a slightly basic buffered solution. In an alternate procedure, proteins were immobilized by non-covalent adsorption on composite sheets that had been treated with a plasma formed from water-saturated argon.

#### Example 2

##### Increasing the Rates of Binding Reactions at a Solid-Phase Support with Sonication: Use of a Low-Power Piezoelectric Buzzer

**[0122]** Streptavidin was immobilized onto chromic acid-oxidized EVA-fibril composite as described in Example 1. A  $\frac{5}{16}$  inch diameter disc cut from this material was placed in the well formed by placing a gasket on a low-power low-frequency acoustic piezoelectric transducer. Treatment of the disc with a solution containing a biotin-labeled  $\alpha$ -Fetoprotein (anti-AFP) antibody (Boehringer-Mannheim, 50  $\mu$ L, 41 nM) led to immobilization of the antibody. The binding reaction was essentially complete in 3 minutes upon sonication by the piezoelectric transducer. The extent of the reaction was determined using a biotin- and TAG1-labeled antibody and measuring bound antibody by ECL. The same reaction took more than 20 minutes when mass-transport occurred through diffusion alone that is, without sonication.

**[0123]** The antibody-coated composite was washed with 50 mM phosphate, pH 7.5. To assay for  $\alpha$ -Fetoprotein (AFP) in a sample, a solution containing a TAG1-labeled secondary antibody directed against AFP (Boehringer-Mannheim, 50  $\mu$ L, 12  $\mu$ g/mL) followed by the sample (10  $\mu$ L) were added to the well. The piezoelectric transducer was used to sonicate the composite and solution for a period of 5 minutes. The disc was washed with phosphate buffer and placed in an electrochemical cell designed for measuring ECL. The cell was filled with ORIGIN Assay Buffer (IGEN, International) and the potential of the composite was scanned from 0 to -0.8 to 1.2 V (vs. Ag/AgCl) at a scan rate of 100 mV/s. The difference between the integrated ECL signal (S) obtained for samples containing known concentrations of AFP and the background signal (B) determined for in the absence of AFP is shown in **FIG. 8** where the signals are provided using a relative scale of intensity). The rate of formation of the sandwich complex was 3-4 times faster when sonication energy was applied (piezoelectric transducer "on") as opposed to when sonication energy was not applied (transducer "off"). Similar results were obtained using a piezoelectric transducer that operated in the ultrasonic frequency range.

#### Example 3

##### Increasing the Rates of Binding Reactions at a Solid-Phase Support with Sonication: Use of an ECL Cell Instrument with an Integrated Piezoelectric Sonication Device

**[0124]** EVA-fibril composite was treated with a water-saturated argon plasma and coated with an anti-AFP anti-

body (Boehringer-Mannheim) as described in Example 1. A 10 $\times$ 15 mm rectangle of the composite was placed in an ECL cell (see **FIG. 6**).

**[0125]** The sample (10  $\mu$ L) and a solution containing a TAG1-labeled anti-AFP antibody (Boehringer-Mannheim, 50  $\mu$ L, 12  $\mu$ g/mL) were combined and introduced into the cell. The binding reaction was allowed to proceed for 3 minutes during which time a piezoelectric transducer (sonication generator) was driven at its resonance frequency (47 KHz) at a power of approximately 2.5 W. The transducer was turned off, the cell was flushed with ORIGIN Assay Buffer (IGEN, International), and the voltage at the composite was ramped from 0 to -0.8 to 1.2 V (vs. Ag/AgCl) at a rate of 0.1 V/s. The difference between the integrated ECL signal (S) obtained for samples containing known concentrations of AFP and the background signal (B) determined for in the absence of AFP is shown in **FIG. 9** (where the signals are provided using a relative scale of intensity). The assay demonstrated a dynamic range of greater than three orders of magnitude and precision of  $\pm 5\%$  or better.

#### Example 4

##### The Binding Kinetics for Formation of a Sandwich Immunocomplex on a Solid-Phase Support: The Effect of Sonication on an AFP Assay

**[0126]** The kinetics of the binding reaction of the AFP assay described in Example 3 were determined by varying the incubation time allowed for the formation of the sandwich immunocomplex on the composite. A sample containing AFP at a concentration of 59 IU/mL was used in these experiments. Two sets of experiments were conducted. In one set of experiments sonication energy was applied (the piezoelectric transducer was activated) during the incubation time for the binding reactions, while in the other set, sonication energy was not applied (the piezoelectric transducer was not activated).

**[0127]** **FIG. 10** shows the intensity of the ECL (on a relative intensity scale) that was measured as a function of the time allowed for the formation of the immunocomplex. The measured ECL signal (for a given incubation time) was larger for those samples that had sonication energy applied during the incubation period than for those samples that did not. Since the magnitude of the measured ECL signal increases with increased binding (to form more sandwich immunocomplexes), these results clearly show that sonication of the assay significantly increases the rate of the binding reactions. Using the slope of the line connecting the first two points as a rough indication of the rate of binding, the rate enhancement attributable to sonication was greater than a factor of 7. The plot also shows that the three-minute assay described in Example 3 is a kinetic assay (in that period of time the binding is approximately one-third to one-half complete); after 10 minutes, the reaction is essentially complete.

#### Example 5

##### Use of Piezoelectric Transducer to Increase the Rate of Mass Transport to and/or from an Electrode During an Electrochemical Reaction: Sonication of Fibril-EVA Composite Electrodes During the Excitation of ECL

**[0128]** An untreated EVA-fibril composite electrode was placed in the ECL cell described in Example 3. The cell was